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1140 W. Orange Grove Ave., Arcadia, California, U.S.A.

OVIPOSITIONAL PREFERENCE TESTS WITH PIERIS

WILLIAM HOVANITZ AND VINCENT C.S. CHANG

California Arboretum Foundation, Arcadia, California, and
Los Angeles State College, Los Angeles, California

PREVIOUS REPORTS BY US (Hovanitz and Chang, 1962, 1963) have described the results of larval selections of various food plants. The present paper continues this series of reports by describing the selections made by the female adults on various food plants for the purpose of egg laying. A similar type of study has been made by Takata (1961) using *Pieris rapae crucivora,* though with somewhat different results. This study will be mentioned below.

METHOD

The ovipositional tests were made in marquisette-covered cages, 18″ x 18″ x 24″ tall. The plants used in each test were living, potted plants grown in the glass house. In any given test, the plants selected were all of about the same size and were relatively small so as to fit in the dimensions of the cage size without severe crowding. The differing shapes, growing habits and leaf sizes of the plants used were variables which could not be controlled. There is no doubt that these factors might have some influence on egg laying of the adult females.

The females were placed in the cages with the respective plants and allowed to mate and to oviposit. The eggs were counted periodically for the totals indicated in the tables. The females used in each cage varied in number from five to fifteen but the average was between five and ten. These were fed honey and water solution in the mornings during the course of each test.

RELATIONSHIP BETWEEN OVIPOSITIONAL PREFERENCE OF THE FEMALE AND THE FOOD PLANT STRAIN OF ITS LARVA

A relationship is apparent between the ovipositional preference of the adult female and the food plant eaten by its larva. This relationship may be seen in table 1 in which strain A, *Pieris napi,* laid more eggs on its ancestral food plant *Dentaria* than on any of the other plants

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Table 1. Ovipositional selections by adults of *Pieris*

A	Species	Mustard	Kale	Nasturtium	Dentaria	Isomeris	Total	Natural food plant
	napi	82 31.66%	43 16.6%	28 10.81%	105 40.54%	1 0.38%	259	Dentaria
B	Species	Mustard	Kale	Nasturtium	Lepidium	Isomeris	Total	Natural food plant
	protodice	305 23.55	12 0.92	6 0.46	536 41.31	436 33.67	1295	Lepidium
C	Species	Mustard	Kale	Nasturtium		Isomeris	Total	Natural food plant
	protodice	417 67.58	41 6.64	3 0.49		156 25.28	617	Mustard

Table 2. Ovipositional selections by adults of *Pieris protodice*
Strain C from wild (Laguna Canyon on mustard), C → D 2 generations on Kale
C → E 2 generations on mustard

Species	Mustard	Kale	Nasturtium	Isomeris	Total	Natural food plant
C protodice	417 67.58%	41 6.64%	3 0.49%	156 25.28%	617	mustard
D protodice	190 26.39	210 29.17	23 3.19	297 41.25	720	
E protodice	378 60.87	43 6.93	1 0.16	199 32.05	621	

tested. Over forty percent were laid on *Dentaria*, about thirty-two percent on black mustard (*Brassica nigra*), about seventeen percent on kale and less than one percent on Isomeris, out of a total of 259.

This relationship is further apparent in the data of strain B, *Pieris protodice* (table 1). A strain of this species from Mono Lake, Calif. where the native food plant is *Lepidium,* laid forty-one percent on *Lepidium,* about twenty-four percent on mustard, about one percent on kale and thirty-four percent on Isomeris, out of a total of 1295. The high percentage on *Isomeris* is interesting in view of the fact that Isomeris does not exist in Mono Lake area.

Another strain of *Pieris protodice* from Laguna Canyon, Orange county, Calif., strain C, where the food plant is black mustard, gave different results. Females of this strain laid nearly sixty-eight percent of their eggs on black mustard, seven percent on kale, less than one percent on nasturtium and twenty-five percent on Isomeris. Unfortunately, there was no Lepidium available for testing with these females.

The two tests with *Pieris protodice* show a quantitative relationship between the selection by the females and the odor of the plants selected. Excluding naturtium, the plants may be arranged in the order Isomeris, *Lepidium,* mustard and kale in the direction from strong odor to weak odor. Females with *Lepidium* ancestry show a higher selection for plants with a stronger odor, and plants with a mustard ancestry show selections favoring a decreased odor requirement. In Table 1, strain B (Lepidium ancestry) shows a selection toward Isomeris of 33 compared with 25 for strain C (mustard ancestry). Likewise, strain B shows only one percent toward kale as compared with seven percent for strain C. The increase in selection of the mustard strain toward mustard (68 percent as compared with 23 percent) is therefore not the whole picture, as comparable changes occur also with the other plants.

CHANGE OF OVIPOSITIONAL PREFERENCES BY FOOD PLANT CHANGES OF THE LARVAE

Changing the food plants fed the larvae has the direct effect of changing the plants selected by the adult females for egg laying. Table 2 shows the effect of splitting strain C (table 1) into two strains, one fed on kale (D) and the other fed on mustard (E). These were grown for two generations on the respective food plants.

The results indicate an increased selection for kale by the kale strain, 29 percent as compared with 7 percent. The strain kept on mustard (E) did not vary greatly from the selections made by the original parents strain (C).

Table 3. Ovipositional selections by Pieris rapae, parent strains, F_1 and F_2. Larvae always fed on food plant of female parent. Parent strains, 10 or more generations on a particular plant.

	Mustard		Kale		Nasturtium		Cleome		Isomeris		Total
Parents											
mustard-strain	1080	86.81%	24	1.92%	38	3.05%	80	6.43%	22	1.70%	1244
Kale-strain	125	18.65	433	64.62	70	10.4	28	4.1	14	2.08	670
F_1											
M ♀ x K ♂	467	34.92	346	25.87	471	35.22	12	0.89	41	3.06	1337
K ♀ x M ♂	263	23.88	328	29.61	466	42.32	35	3.17	11	0.99	1101
F_2											
M ♀ x K ♂	969	47.57	644	31.60	344	16.40			90	4.42	2037
K ♀ x M ♂	567	29.50	783	40.70	486	25.29			86	4.47	1922

Table 4. Ovipositional selections by P. rapae reared on Isomeris for a given number of generations, 1961 tests.

Generation	Mustard		Kale		Isomeris		Nasturtium		Total
Second	148	47.20%	91	29.1%	1	3.2 %	73	23.3 %	313
Fourth	237	54.50	91	20.0	162	23.9	10	1.6	500

A similar relationship has been found with *Pieris rapae* (Table 3). A strain kept for 10 or more generations on mustard in the laboratory was compared with a strain grown an equal length of time on kale. The mustard bred strain showed a selection of 87 percent on mustard as compared with a selection by the kale strain of only 19 percent on that plant; the kale strain adults selected kale 65 percent of the time as compared with only 2 percent on kale from the mustard strain.

Pieris rapae, which have been switched over from kale or mustard to Isomeris, also show an increased preference for the latter plant but the selections take a lot longer period of time to become effective. It has previously been shown that larvae of *Pieris rapae* grown on kale for the first time have a high mortality rate approaching the lethal condition. However, after a number of generations they are able to survive satisfactorily on the new food plant. Ovipositional tests (Table 4) of *Pieris rapae* reared on Isomeris show that adults from the second generation of larvae fed on Isomeris show little change in their selection. Most eggs are still laid on mustard with a high proportion on kale and nasturtium. In the fourth generation however, there are 24 percent of the eggs laid on Isomeris and a corresponding drop in those laid on nasturtium. A more complete series of tests of this sort is shown in Table 5 which will be discussed below.

GENETIC CHANGE OF OVIPOSITIONAL PREFERENCES AS SHOWN BY INTERSTRAIN CROSSES

The genetic significance of the changes in ovipositional preferences as created by larval feeding habits are shown by crosses involving parent strains, one of which has been adapted to kale and the other to mustard (Table 3). The parent strains have been grown for ten or more generations on their respective food plants and show a high ovipositional selection for their own larval food plants (fig. 1).

The F_1 of the cross kale-strain female X mustard-strain male was grown on kale and the reciprocal cross was grown on mustard. Ovipositional preferences in the first of these two crosses indicate that the F_1 adults, even though fed on kale as larvae, did not select kale to the extent of the kale parental strain. There was a drop in selection of kale from 65 percent to 30 percent. The increase appears to have all gone to the selection of nasturtium (fig. 2).

In the reciprocal cross, the F_1 larvae were grown on mustard. The adults from these larvae dropped in their selection of mustard from 87 perecent to 35 percent. The increase in this case was not only toward nasturtium but also toward kale.

It is clear from these crosses that the genes for the inheritance of food plant preferences by the adult for ovipositional purposes is transmitted through the male as well as through the female. Indications

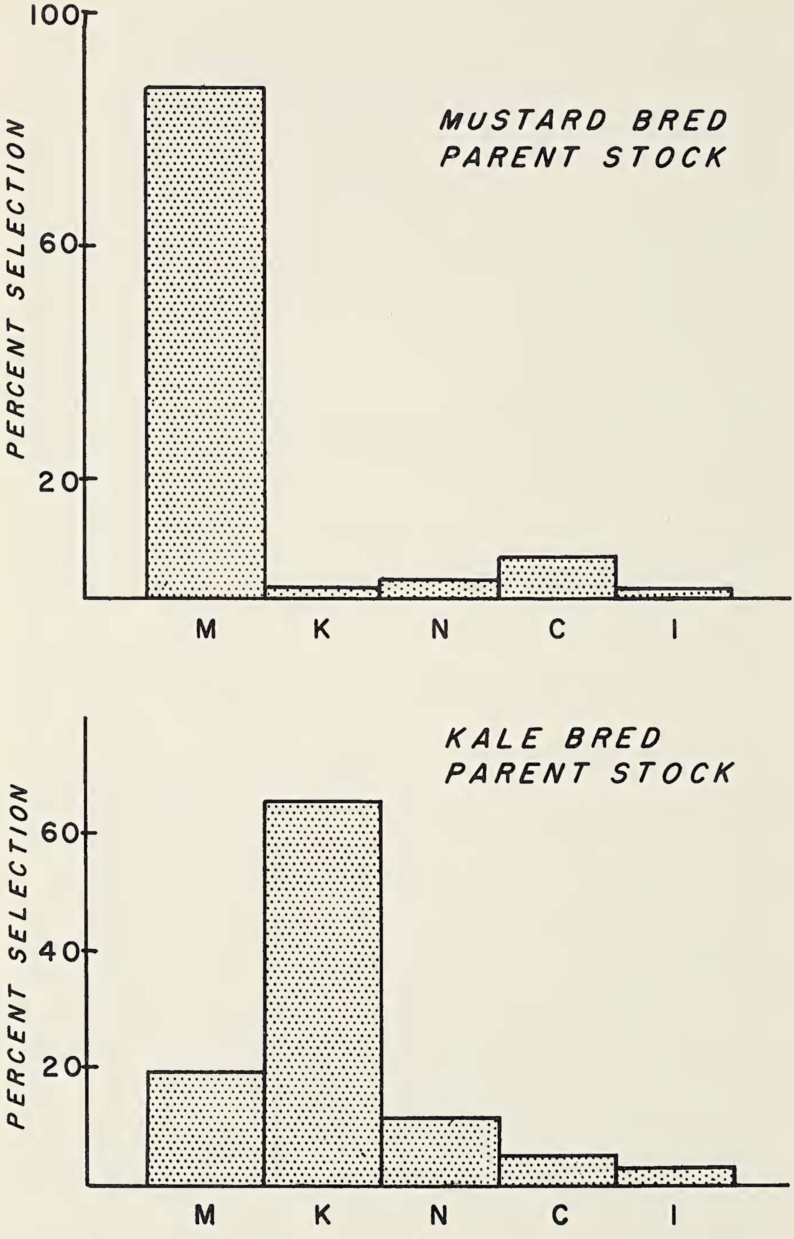

Fig. 1. Oviposition selection by females of *Pieris rapae* for the plant on which the strain has been maintained for more than 10 generations.

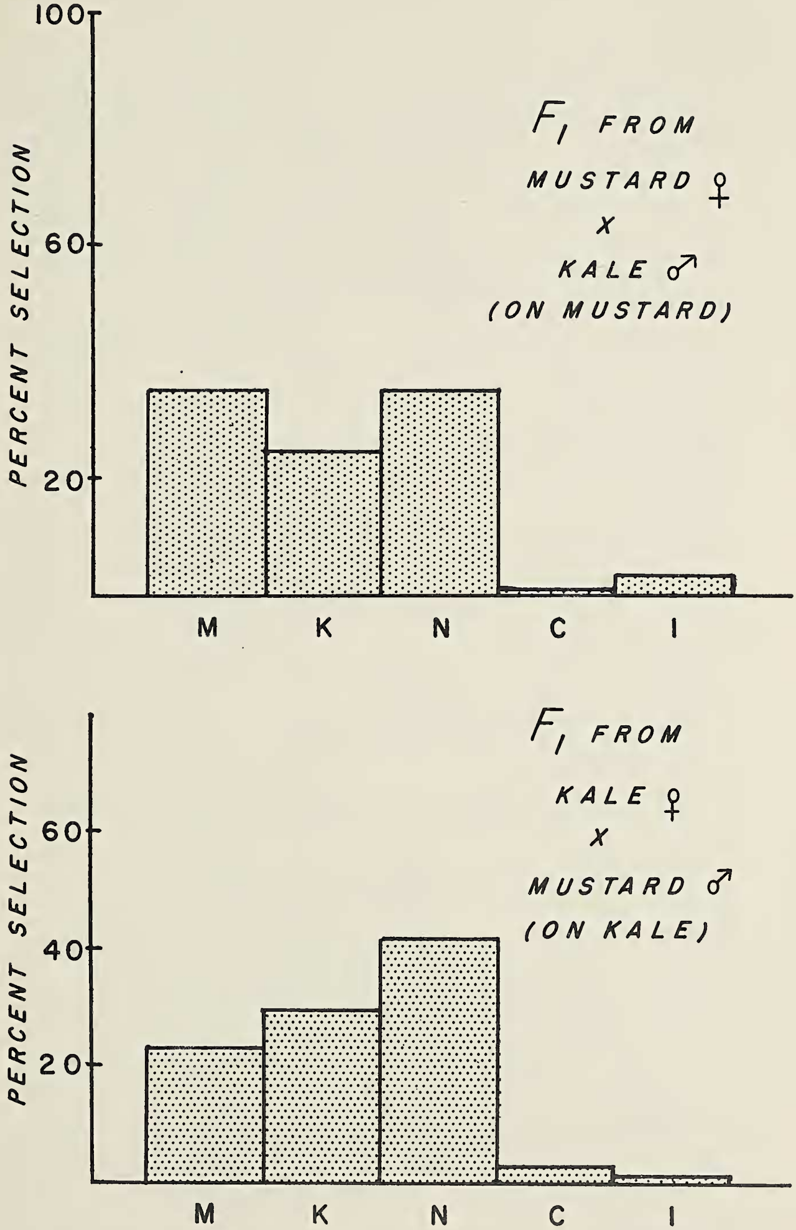

Fig. 2. Oviposition selection by females of *Pieris rapae* produced by crossing mustard and kale strains to obtain the F_1.

Table 5. Ovipositional selections by adults of P. rapae grown on nasturtium for a given number of generations. (former plant, kale)

Generation	Mustard		Kale		Nasturtium		Isomeris		Total
1	546	36.27%	662	43.98%	297	19.73%	0	0	1505
3	100	8.31	555	46.09	463	38.45	86	7.14 %	1204
5	97	26.50	106	28.96	163	44.53	0	0	366
7	186	32.12	113	19.51	194	33.50	86	14.85	579
8	16	17.02	16	17.02	35	37.23	27	28.72	94

Table 6. Ovipositional selections by adults of P. rapae grown on Isomeris for a given number of generations. (former plant, kale)

Generation	Mustard		Kale		Nasturtium		Isomeris		Total
1	330	45.70%	299	41.41%	61	8.44 %	32	4.43 %	722
3	55	28.94	108	56.84	5	2.63	22	11.57	190
5	333	34.43	481	49.74	77	7.90	76	7.85	967
7	237	26.93	307	34.88	47	5.34	289	32.84	880
9	76	30.27	90	35.80	17	6.77	68	27.09	251
11	192	26.32	363	49.59	38	5.15	139	18.99	732

Table 7. Ovipositional selections by adults of P. rapae grown on kale for a given number of generations. (former plant, kale)

Generation	Mustard		Kale		Nasturtium		Isomeris		Total
1	340	42.55 %	389	48.88%	40	5.04%	27	3.39%	796
3	204	18.08	778	68.79	95	8.42	53	4.70	1128
5	402	19.97	1397	69.39	152	7.55	62	3.07	2013
7	91	27.00	208	61.72	30	8.90	8	2.37	337
8	100	36.90	102	37.63	31	11.44	38	14.02	271
9	128	18.57	299	43.40	109	15.82	153	22.21	689
10	341	41.13	375	45.23	45	5.40	68	8.20	829
12	627	23.92	1574	60.07	54	2.06	367	14.00	2622

Table 8. Ovipositional preference of P. protodice females from Palm Springs (area of Isomeris).

Mustard		Kale		Nasturtium		Isomeris		Total
142	41.16%	1	0.29%	0	0	202	58.55%	345

that these traits may be maternally inherited are therefore not wholly if at all true.

The F_2 of the mustard female F_1 and the reciprocal shows a somewhat similiar selection pattern as the F_1, with the exception that the selection of nasturtium has been reduced in favor of both mustard and kale (fig. 3).

RATE OF CHANGE OF OVIPOSITIONAL PREFERENCES OVER A PERIOD OF SEVERAL GENERATIONS

The rate of change of ovipositional preferences during the course of several generations has been studied in three series, one in which the larvae have been bred on nasturtium, one on Isomeris and one on kale. These data are indicated in tables 5, 6 and 7.

Nasturtium is not a normal food plant of *Pieris rapae*, but colonies of the insect have become adapted to the plant both in Europe and in the United States. Larvae from a strain previously fed on kale were transferred to nasturtium and maintained on that plant for eight generations. Adults from some of these generations were tested for their preferences toward kale, mustard, nasturtium and Isomeris. The normal selection of kale strain adults to Isomeris is about 10 percent (Table 3). The first generation after the larvae were fed nasturtium, the selection went up to 20 percent, the third generation 38 percent, the fifth generation 45 percent, the seventh generation 34 percent and the eighth generation 37 percent. The lack of any change above 34 percent may mean that this is the best possible selection that can be obtained after any amount of selection.

It is significant that during the course of these selections for nasturtium, selection for kale went progressively down and that at the same time selection for Isomeris went progressively up. As has been previously pointed out, this result may be due to the stronger odoriferous substances present in nasturtium and Isomeris, absent in kale.

Isomeris is not known to be a wild food plant for *Pieris rapae* anywhere. Larvae are, however, able to survive on this plant, especially after conditioning. Larvae from a strain fed previously on kale were trensferred to Isomeris and maintained on this plant for eleven generations. The normal selection of kale strain adults to Isomeris is about 2 percent (Table 3). The first generation of adults from larvae fed on Isomeris selected this plant 4 percent of the time, the third generation 12 percent of the time, the fifth generation 8 percent of the time, the seventh generation 32 percent of the time, the ninth generation 27 percent of the time and the eleventh generation 19 percent of the time (fig 5). A significant increase is apparent, though the variation is somewhat great, and this increase is additive for several generations. It is of interest that the selection of Isomeris, though

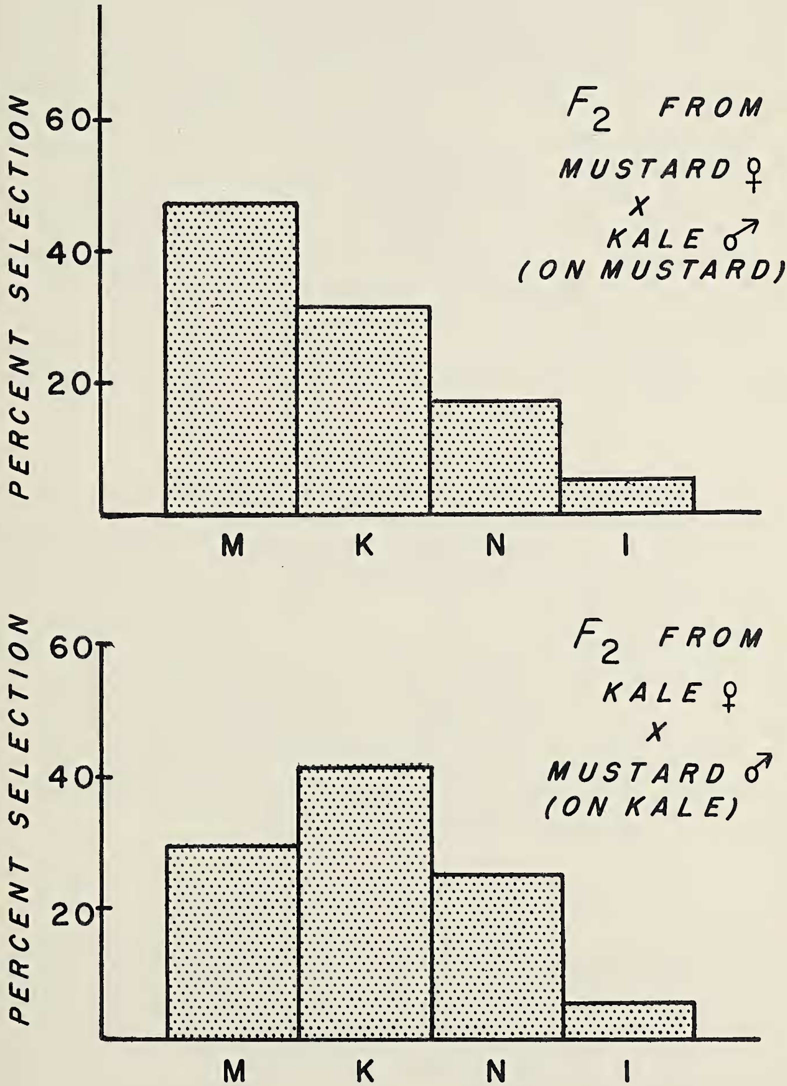

Fig. 3. Oviposition selection by females of *Pieris rapae* produced by crossing the F_1 individuals indicated in fig. 2.

increased, is never greater than that for kale and only comes close to duplicating the selections for mustard.

Strains grown on kale for twelve generations, after originating from wild individuals growing on mustard, were tested in the third experiment. A mustard strain selected kale about two percent of the time. After one generation on kale, the selection of kale was 48 percent, after three generations 69 percent, five generations 69 percent, seven generations 62 percent, eight generations 38 percent, nine generations 43 percent, ten generations 45 percent and twelve generations 60 percent (fig. 6). Again there is no doubt of the increased rate of selection of the larval food plant by the adults for the first few generations though there are somewhat erratic results thereafter due to unknown causes. In fact after three generations the selections of kale were as good as is expected in a strain kept permanently on kale (Table 3).

This experiment indicates that adaptation to kale is much faster than to plants such as Isomeris and nasturtium, and is also much more effective.

ADDENDUM

The following data were made available after the previous portion of this paper was written. Adults of *Pieris protodice* were collected in the vicinity of Palm Springs, in an area typical of the Coachella desert. As a result it cannot be anticipated that the cruciferous plants typical of coastal California were present. Instead, the bushes of Isomeris are the most abundant plants of this group. These females were allowed to oviposit in tests as the preceding, with the result that Isomeris was the plant most commonly selected (59 percent) with mustard in abundance (41 percent) (Table 8). These data compare with *P. protodice* selections in an area where mustard is the wild food plant as follows: Isomeris 25 percent instead of 59 percent, and mustard 67 percent instead of 41 percent.

DISCUSSION AND CONCLUSIONS

These results of ovipositional experiments illustrate the same events as have been shown previously for *larval* selections of food plants, namely that previous exposure to a plant is correlated with the selection of that plant by the particular insect. This increase of selection occurs not only in the first generation of exposure, but also after subsequent generations of exposure. It is easier to change the selection of Pieris toward kale than it is toward Isomeris or nasturtium. This is true both with respect to the speed with which the change is made as well as in the extent to which the change occurs.

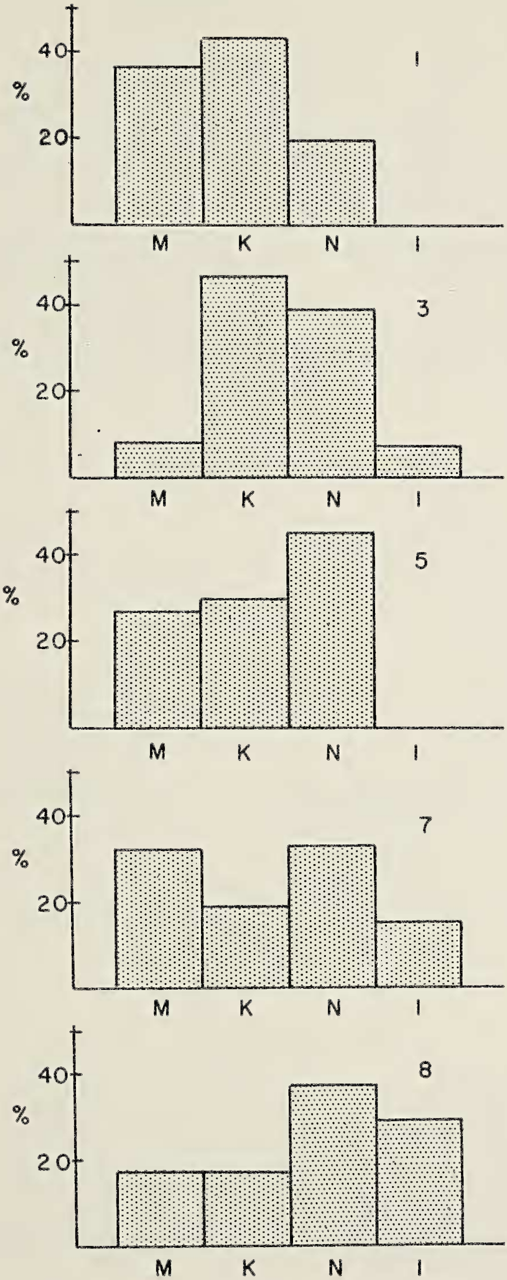

Fig. 4. The change in ovipositional selection by adults of *Pieris rapae* after 8 generations of growing on nasturtium.

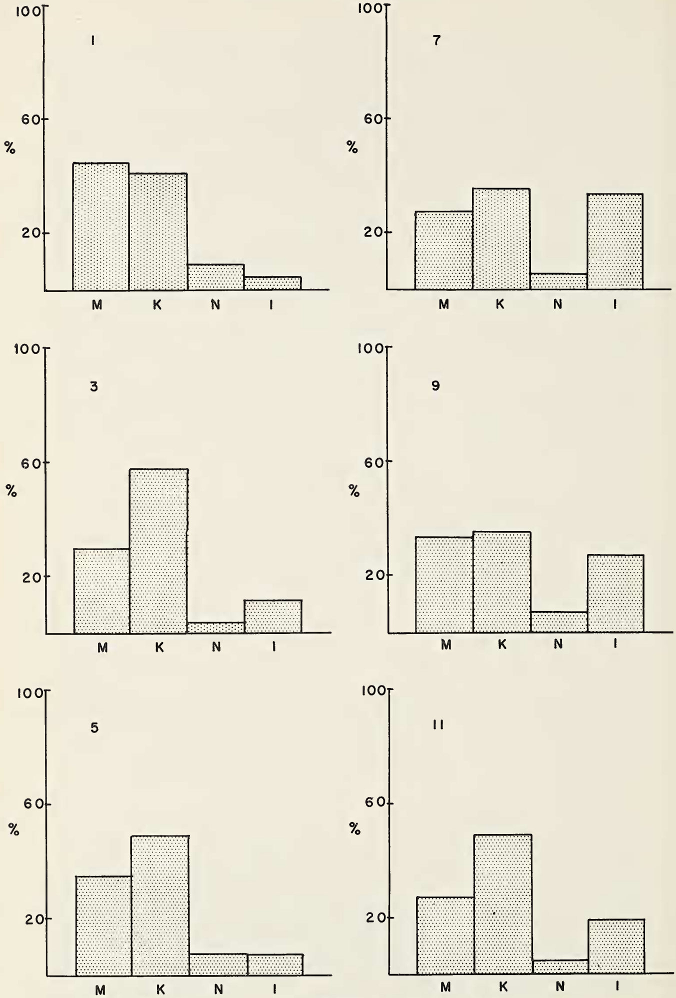

Fig. 5. The change in ovipositional selection by adults of *Pieris rapae* after eleven generations of growing on Isomeris.

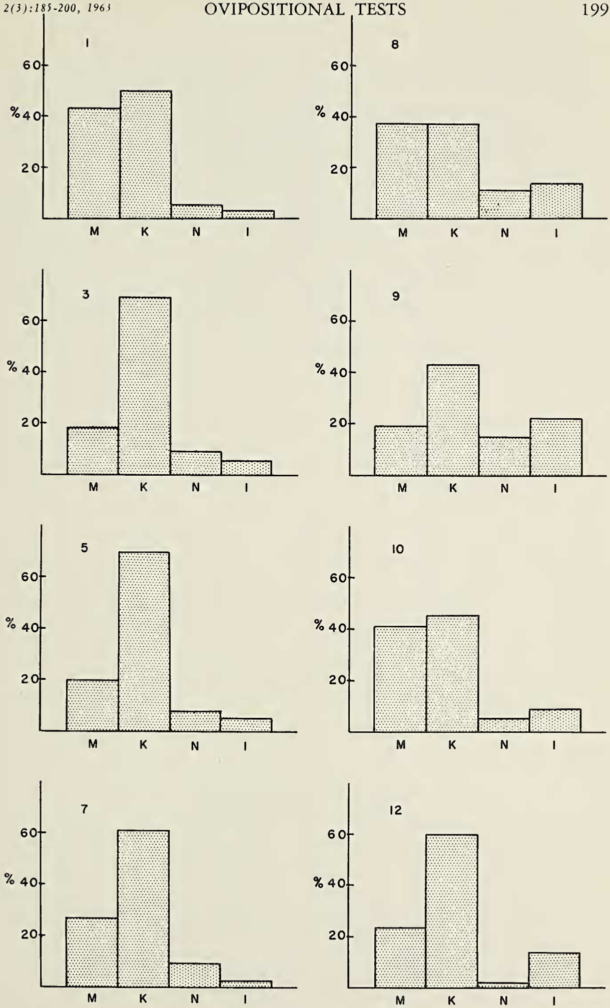

Fig. 6. The change in ovipositional selection by adults of *Pieris rapae* after twelve generations of growing on kale.

The heredity of the food plant selections has been tested by both F_1 and F_2 crosses. The results indicate that the F_1 selections are intermediate between the parental selections and that the F_2 is also, but the latter tends to match the food plant on which the F_2 larvae are fed. An unknown cause accounts for a heavy increase in selection of nasturtium in the F_1 which is not so apparent in the F_2. The gene or genes for the food plant specificities is clearly carried both by the males as well as by the females and there is no sign of maternal inheritance, except a psuedo-maternal effect which is imposed upon the progeny by the food directly eaten by them.

These results are directly opposed to those of Takata (1961) in which he says: "If the larvae were reared with cabbage for successively many generations, the emerged butterflies tended more and more to avoid cabbage as their ovipositing plant, as the successive life on cabbage was lengthened . . . The direction of the change was quite inverse to that of the larval food preference, which was shown by that the larvae preferred the host plant on which they and their ancestors had been fed." No reason can be given for the difference between his results and ours, nor is their any clue as to what it might be with the one exception: In our own experiments, more than two plants were available both for larval selections as well as for adult selections. It was possible in our experiments therefore for the adults to select some plant other than the two being tested in case neither of the original two were satisfactory. As an example of this phenomenon, in the F_1 of the mustard-kale strain crosses, the greatest proportion of the adults selected nasturtium rather than either mustard or kale. Had nasturtium not been available, the adults would have had to choose between two plants neither of which was preferred. Something of this sort might explain the results of Takata.

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